In addition to polarizing and orienting their axes, migrating cells also need to create forces for movement, build new cell adhesion sites, remove old ones, and deliver secretory vesicles to the right place. It is likely that these cytoskeletal elements are regulated and organized in different ways in different parts of the cell because all these steps require actin and/or microtubules. The present paper not only contributes to our understanding of cytoskeletal organization in migrating cells, but also provides an important handle for future studies of these fascinating issues.

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Selected Reading

Bashour, A.M., Fullerton, A.T., Hart, M.J., and Bloom, G.S. (1997). J. Cell Biol. *137*, 1555–1566.

Brunner, D., and Nurse, P. (2000). Cell *102*, 695–704.

Fukata, M., Kuroda, S., Fujii, K., Nakamura, T., Shoji, I., Matsuura, Y., Okawa, K., Iwamatsu, A., Kikuchi, A., and Kaibuchi, K. (1997). J. Biol. Chem. *272*, 29579–29583.

Fukata, M., Watanabe, T., Noritake, J., Nakagawa, M., Yamaga, M., Kuroda, S., Matsuura, Y., Iwamatsu, A., Perez, F., and Kaibuchi, K. (2002). Cell *109*, 873–885.

Goode, B.L., Drubin, D.G., and Barnes, G. (2000). Curr. Opin. Cell Biol. *12*, 63–71.

Gundersen, G.G. (2002). Nat. Rev. Mol. Cell Biol. *3*, 296–304.

Palazzo, A.F., Cook, T.A., Alberts, A.S., and Gundersen, G.G. (2001). Nat. Cell Biol. *3*, 723–729.

Perez, F., Diamantopoulos, G.S., Stalder, R., and Kreis, T.E. (1999). Cell *96*, 517–527.

Ridley, A.J. (2001). J. Cell Sci. *114*, 2713–2722.

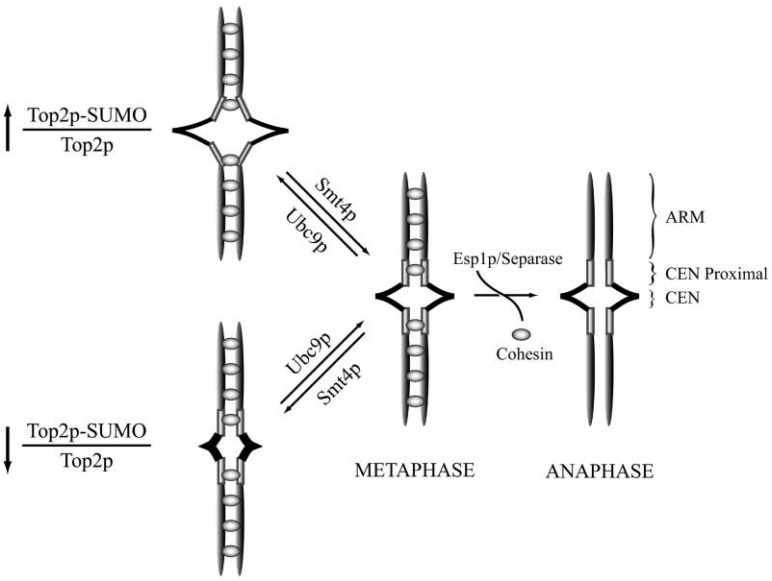
Wittmann, T., and Waterman-Storer, C.M. (2001). J. Cell Sci. *114*, 3795–3803.

Top-SUMO Wrestles Centromeric Cohesion

Sister chromatid cohesion at the centromere is distinct from cohesion at the chromosome arms. In the June issue of *Molecular Cell*, Bachant et al. have shown that centromeric cohesion in budding yeast is specifically regulated by SUMO-1 modification of Topoisomerase II.

The faithful transmission of genetic material requires a series of finely orchestrated cell cycle events that ensure accurate chromosome segregation. Critical to this process is the establishment, preservation, and timely dissolution of cohesion between sister chromatids (for a review, see Nasmyth, 2001). Globally, cohesion preserves the relationship between sisters throughout chromosome condensation and alignment on the mitotic spindle so that each daughter cell receives a single copy of every chromosome. Looking more closely, however, it is clear that not all cohesion is created equal. In particular, centromeric chromatin appears to have unique cohesion properties. For multicellular eukaryotes, this centric specialization is manifest in the way the essential protein mediator of sister chromatid cohesion, the cohesin complex, is removed from the sister chromatids. Although the bulk of the cohesin complex is unloaded from the chromosome arms during prophase in a polo-like kinase-dependent fashion, chromosome segregation cannot take place until centromeric cohesion is relieved at the metaphase to anaphase transition. The release of centromeric cohesion occurs via cleavage of the cohesin subunit Mcd1p/Scc1p, a process that involves activation of the protease Esp1p/Separase. This evolutionarily conserved proteolytic event appears to be the exclusive means by which the budding yeast achieves cohesin removal along the length of the chromosomes. However, several observations indicate that the centromeric region of this model organism also has distinct cohesion qualities. Upon achieving bipolar attachment to the spindle, the tension generated across budding yeast sister centromeres causes them to undergo precocious separation while the chromosome arms remain intimately associated. This precocious separation occurs despite enrichment of the cohesin complex at centromeric regions, suggesting that the cohesive structure of centromeric chromatin is designed for elasticity to accommodate the dynamic forces of the spindle. Despite this specialization of centromeric cohesion, the molecular determinants that distinguish mitotic centromeric cohesion from arm cohesion in budding yeast have yet to be described.

The Elledge lab has now identified the small ubiquitin-related modifier Smt3p/SUMO-1 as the first factor that contributes specifically to the architecture of centromeric cohesion (Bachant et al., 2002). Unlike ubiquitylation, the addition of Smt3p (sumoylation) is not known to target proteins for degradation. Rather, the Smt3p/SUMO-1 modification antagonizes ubiquitin-dependent degradation, regulates protein-protein interactions, and alters subcellular localization (for a review, see Melchior, 2000). The link between Smt3p/SUMO-1 and centromeric cohesion developed out of an observation made while visualizing cells harboring a mutation in the Smt3p/SUMO-1 isopeptidase Smt4p, a protein responsible for cleaving Smt3p/SUMO-1 from modified substrates (Li and Hochstrasser, 2000). Although centromeric cohesion is so robust that no defect was initially seen in normally cycling cells, analysis at a mitotic arrest uncovered a phenotype in which the DNA is extensively stretched along the spindle axis. This phenotype differs from mutants in the cohesin complex where the DNA mass becomes fully, but inappropriately, separated. Careful examination of sister chromatid separation at



The Sumoylation of Topoisomerase II Regulates Centromeric Cohesion

The ratio of SUMO-modified Top2p to unmodified Top2p is maintained by the balance of Ubc9p conjugating and Smt4p deconjugating activities. When the ratio of modified to unmodified Top2p increases, the cohesion between CEN and CEN-proximal sites on the chromosome decreases (top left). Conversely, when the relative concentration of the modified Top2p population decreases, centromeric cohesion increases (bottom left). This regulation is independent of Esp1p/Separase-mediated removal of the cohesin complex at the metaphase to anaphase transition (right).

a series of chromosomal positions demonstrated that cohesion at centromere and centromere-proximal regions is sensitive to Smt4p function and that this effect is independent of the centromere localization of Mcd1p/Scc1p cohesin (see Figure). Strikingly, the centric specificity is not simply due to the tension generated across sister centromeres. In *smt4* mutant cells treated with the microtubule depolymerizing drug nocodazole, there is increased centromere separation even in the absence of spindle tension. This exciting result provides the first evidence for the specific molecular regulation of centromeric cohesion.

Both *SMT3* and *SMT4* were originally isolated in a screen for high copy suppressors of mutations in the centromere binding protein Mif2p/Cenp-C (Meluh and Koshland, 1995). In addition, an allele of *smt3* was identified in a microscopy-based screen for mutants defective in sister chromatid separation. Taken together with these new *smt4* results, one can envision that the level of Smt3p/SUMO-1 conjugation of relevant targets may function as a rheostat to modulate centromeric cohesion. When there is a deficiency in Smt3p/SUMO-1 modification, centromeric cohesion strengthens, leading to a failure in appropriate sister centromere separation. Conversely, the untimely conjugation of Smt3p/SUMO-1 reduces centromeric cohesion, resulting in increased precocious sister centromere separation.

What Smt3p/SUMO-1-modified proteins are relevant to centromeric cohesion? Although Smt3p/SUMO-1 localizes to chromatin, to date, only the bud neck-associated mitotic septins have been confirmed as conjugates in vivo (Johnson and Blobel, 1999). Bachant et al. have now identified Topoisomerase II (Top2p) as the first nuclear Smt3p/SUMO-1 target in budding yeast, a modification conserved in human type II topoisomerases (Mao et al., 2000). Furthermore, they have shown that it is the deconjugation of Top2p-Smt3p/SUMO-1 by Smt4p that helps maintain cohesion at the centromere. This is clearly demonstrated by the ability of mutant *top2* lacking Smt3p/SUMO-1 modification sites (*top2-S*umo *N*o-*M*ore, *top2-SNM*) to partially suppress the *smt4* centromeric cohesion defect when it is the sole copy of *TOP2*. However, the partial suppression suggests that Top2p is not the only sumoylated protein mediating centromeric cohesion, and it will be important to identify additional targets.

It is somewhat surprising that Top2p contributes to such a locally specific process, given its wide distribution on DNA and its multiple roles in regulating global chromosome structure (for a review, see Wang, 2002). Most likely it is a subpopulation of cellular Top2p that participates in centromeric cohesion. Does sumoylation influence the localization of a Top2p pool? In mammalian cell culture, a subset of Top2p is localized to the centromeres of mitotic chromosomes, though the basis for this localization is not known (Rattner et al., 1996; Sumner, 1996). Alternatively, is the activity of a pool of Top2p regulated specifically by sumoylation? Though the *top2-SNM* allele can carry out the essential function of decatenating topologically linked sister chromatids, the Smt3p/SUMO-1-modified form has not yet been assayed for enzymatic activity. It is possible that sumoylation of Top2p biases its activity to transform localized centromere topology and thus oppose the centromeric cohesion mediated by cohesin complexes.

While the precise mechanism by which Top2p-Smt3p/SUMO-1 regulates cohesion at the centromere remains mysterious, the work of Bachant et al. provides an intriguing start to understanding the unique properties of centromeric cohesion in budding yeast and possibly all eukaryotes.

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Selected Reading

Bachant, J., Alcasabas, A., Blat, Y., Kleckner, N., and Elledge, S.J. (2002). Mol. Cell *9*, 1169–1182.

Johnson, E.S., and Blobel, G. (1999). J. Cell Biol. *147*, 981–993.

Li, S.-J., and Hochstrasser, M. (2000). Mol. Cell. Biol. *20*, 2367–2377.

Mao, Y., Sun, M., Desai, S.D., and Liu, L.F. (2000). Proc. Natl. Acad. Sci. USA *97*, 4046–4051.

Melchior, F. (2000). Annu. Rev. Cell Dev. Biol. *16*, 591–626.

Meluh, P.B., and Koshland, D. (1995). Mol. Biol. Cell *6*, 793–807.

Nasmyth, K. (2001). Annu. Rev. Genet. *35*, 673–745.

Rattner, J.B., Hendzel, M.J., Furbee, C.S., Muller, M.T., and Bazett-Jones, D.P. (1996). J. Cell Biol. *134*, 1097–1107.

Sumner, A.T. (1996). Chromosome Res. *4*, 5–14.

Wang, J.C. (2002). Nat. Rev. Mol. Cell Biol. *3*, 430–440.

Genetics Leads the Way to the Accomplices of Presenilins

Presenilins mediate the γ-secretase cleavage of Notch transmembrane receptors as well as the transmembrane β-amyloid precursor protein (βAPP), but they are not thought to accomplish this alone. Recent genetic screens in *C. elegans*, presented in this issue of *Developmental Cell*, identify two genes that are essential to γ-secretase activity and may interact with presenilins.

In 1995, presenilin proteins came in to the limelight on two fronts: association with familial Alzheimer's disease and involvement in Notch signaling pathways in model systems (Levitan and Greenwald, 1995; Sherrington et al., 1995). Since then, active research in both fields has led to a unifying model in which integral membrane presenilins are responsible for the γ-secretase activity that cleaves β-amyloid precursor protein (βAPP) and Notch within their transmembrane domains. This proteolytic event is of major interest for two reasons: first, defects in this event result in the pathogenic accumulation of Aβ peptides in Alzheimer's patients, and second, it is this step during Notch signal transduction that releases the Notch intracellular domain from the membrane, allowing it to move to the nucleus and regulate transcription. Although abundant evidence suggests that the active site of γ-secretase resides in the presenilin protein itself, the protein alone is not enzymatically active unless isolated as part of a large multiprotein complex. For this reason, significant attention is currently focused on deciphering the identity and function of the additional components of the presenilin complex.

Currently, the only other known component of presenilin complexes is the type I transmembrane protein APH-2/nicastrin, identified biochemically in immunoprecipitates of presenilins from human cells (Yu et al., 2000). A critical role for APH-2/nicastrin in the Notch signaling pathway had been demonstrated by its discovery in *C. elegans* as a mutant that shows developmental defects identical to those caused by mutations in the Notch receptor itself or in presenilins (Goutte et al., 2000). Further experiments performed in human cells and *Drosophila* have now demonstrated clearly that the APH-2/nicastrin protein is an essential component of the γ-secretase complex that acts to cleave Notch receptors as well as βAPP (Chung and Struhl, 2001; Esler et al., 2002; Hu et al., 2002; Lopez-Schier and St Johnston, 2002). The pursuit of mutations that cause Notch signaling defects has recently yielded yet another essential player in this pathway: the predicted seven-pass transmembrane protein APH-1 (Goutte et al., 2002). Defective APH-2/nicastrin localization in *aph-1* mutants hints at a possible connection to γ-secretase activity.

In a beautiful demonstration of the power of genetic screens, Francis et al. in this issue of *Developmental Cell* report the isolation of two genes in *C. elegans*, *aph-1* and *pen-2*, that interact genetically with presenilins and play a critical role in γ-secretase activity (Francis et al., 2002). Worms that are defective in both presenilin genes, *hop-1* and *sel-12*, display all the hallmark Notch signaling defects: maternal-effect embryonic lethality, aberrant somatic gonad and vulval development, and germline sterility. However, single *sel-12* or *hop-1* mutants are fully viable and fertile. Francis et al. searched for mutations that would act synergistically with a *sel-12* mutation to cause germline sterility. As expected, their screen yielded new alleles of *hop-1*, but they also discovered mutations in two additional genes. One of these genes was *aph-1* and the other was a new gene, which the authors call *pen-2* (*presenilin enhancer*), encoding a small conserved protein with two predicted transmembrane domains. Although the authors isolated these genes in a sensitized genetic background, mutations in either gene alone cause two bona fide Notch phenotypes: maternal-effect embryonic lethality and defective vulval development, suggesting that each gene has an essential function in mediating at least some Notch signaling events. It is possible that the APH-1 and PEN-2 proteins are obligate components of all Notch signaling events in *C. elegans*; however, demonstration of their requirement in later developmental events is hampered by the difficulty of obtaining animals lacking both maternal and zygotic gene products.

Ligand binding to the Notch receptor stimulates two successive cleavages of Notch. The first, at the S2 site, releases the extracellular domain. The remaining membrane-tethered Notch fragment then becomes a substrate for the presenilin-mediated cleavage at the S3 site within the transmembrane domain. This cleavage releases the Notch intracellular domain, allowing its transit to the nucleus. In order to delineate which step in the Notch signaling pathway requires *aph-1* and *pen-2* function, Francis et al. used two constitutive versions of Notch: a gain-of-function derivative of LIN-12 (one of two Notch receptors in *C. elegans*), which is still membrane anchored and therefore dependent on S3 cleavage, and a truncated LIN-12, consisting of only the intracellular portion of Notch, which therefore does not require S3 cleavage to elicit signal transduction. They found that the membrane-anchored form required *aph-1*